ble in theory but would require a logarithmically increasing dilution rate.

Summary

An apparatus is described for precise measurement of growth rate of an alga as a function of intensity and intermittency of illumination. Dilution of a culture is controlled by a photometric device to just such a rate as will maintain constant density of population and balance the growth rate. Maintenance of constant volume by an automatic siphon allows direct application of the differential form of the growth equation in evaluating specific growth rate. The relationships of the new apparatus to other steady-state growth devices are discussed.

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GROWTH RATE OF CHLORELLA IN FLASHING LIGHT[1]

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Studies on the mass culture of algae under sunlight conditions have demonstrated that the most important factor limiting the yield per unit area of exposed surface lies in the characteristic of light saturation (12). Cells at the front surface of a culture use the very high light intensity of sunlight with low efficiency; at the same time cells at the back surface of a dense culture may receive no light at all. In theoretical studies of effects of intermittent light on photosynthesis it has been shown that light of high intensity may be used with high efficiency if presented in short flashes separated by long dark periods. One anticipates that some similar effect will hold for the total growth process as well as for photosynthesis alone. If, by turbulence of culture suspension, individual algal cells are moved back and forth between the high intensity of the front surface and the darkness of the back surface, an improvement in over-all efficiency of light utilization by the culture might be effected.

Reported herein is an investigation of the characteristics of growth of a representative alga, *Chlorella pyrenoidosa*, in intermittent light. In order to obtain interpretable results, rate of growth has been studied in thin layers of culture suspension of such low population density that mutual shading effects of the cells are minimized. Intermittent illumination of measurable characteristics has been provided by a mechanical optical system. As a secondary objective it has been necessary to determine also the characteristics of the light intensity curve for growth in continuous illumination.

Materials and Methods

The growth rate at each light intensity or intermittency regimen was determined by a separate experiment. Cells harvested from a continuous-culture apparatus were centrifuged out and resuspended in a ¼-strength EDTA Knops solution (13) to a population density which gave a 50 % transmission in an Evelyn colorimeter with 600 mμ glter. In the growth chamber this suspension had a transmission of 81 % as measured with a fully-illuminated photocell placed behind the suspension. Constancy of the photometric control was checked during and at the end of each experiment by readings of transmission of the suspension in the Evelyn colorimeter; all such readings fell within the range of 48–52 %. In each experiment the specific growth rate, k, was evaluated in $\log_e$ units per day as described in the preceding paper. The temperature used throughout was 25°C.

Intensity Measurement: Measurements of light intensity at the position of the growth chamber were made with a large surface Moll thermopile with a compensated Aryton shunt and Rubicon Type T galvanometer. It was calibrated without a shielding window against an NBS standard lamp. For subsequent measurements the thermopile was used with a thin glass shielding window. Estimate of the intensity of visible radiation was obtained by use of a Jena RG8 filter which transmits the near infrared and has a sharp cut-off at 7000 Å (fig 1). Readings with the thermopile were taken alternately with the system open, with the RG8 filter, and with the system closed by a shutter. (For positions of the shutter and filter in the optical system see figure 2 of the preceding paper.) Total deflection with the system open, minus deflection with the RG8 filter was taken as the measure of visible radiation. The procedure is similar to that used by Kok (8). A series of check readings made with a calibrated Weston 603 Illumination meter showed good linearity with the thermopile and RG8

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filter method at lower light intensities. In the intensity data to be presented below, 41 erg/cm²-sec is equivalent to 1.0 foot candle.

LIGHT INTENSITY CONTROL: The light source was a 1000-watt, 120-volt projection lamp operated at 100 volts ± 0.5 volt from a Raytheon voltage stabilizer and variable transformer. In the experiments with intermittent light the voltage was increased to 105 volts in order to obtain a somewhat higher intensity. This makes a small but not important change in the spectral quality of the light used in the two series of experiments. The intensity for each experiment was taken as the average of values obtained at the beginning and end which, except in two very long experiments, were within 2 % of the average.

For study of growth rate as a function of intensity of continuous illumination the intensity was varied by use of Wratten neutral filters or copper screens in position F of the optical system. Lower intensities required addition of a ground glass diffusion plate in position S. Transmission versus wave length curves of the neutral filters, the RG8 filter, and the thermopile window, as determined with a Beckman spectrophotometer, are presented in figure 1. The neutral filters are reasonably flat in the 5000 to 7000 Å region which contains 86 % of the visible radiation used (fig 2).

SPECTRAL CHARACTER OF THE ILLUMINATION: The spectral distribution of the illumination used is presented in figure 2. Curve A is the relative energy output of the lamp operated at 100 volts, color temperature 2990°K, with corrections for tungsten emissivity (data kindly supplied by Dr. H. Haynes of General Electric). Curve B is the relative energy output of the lamp transmitted by 23.5 cm of water as calculated from the data of Collins (3). Curve B represents the spectral distribution of the illumination seen by the algal suspension. Curve C is the fraction of the total illumination (curve B) transmitted by the RG8 filter.

The method of estimating intensity in the visible region described above, effectively subtracts the area under curve C (transmitted by the RG8) from the area under curve B (total illumination). Actually

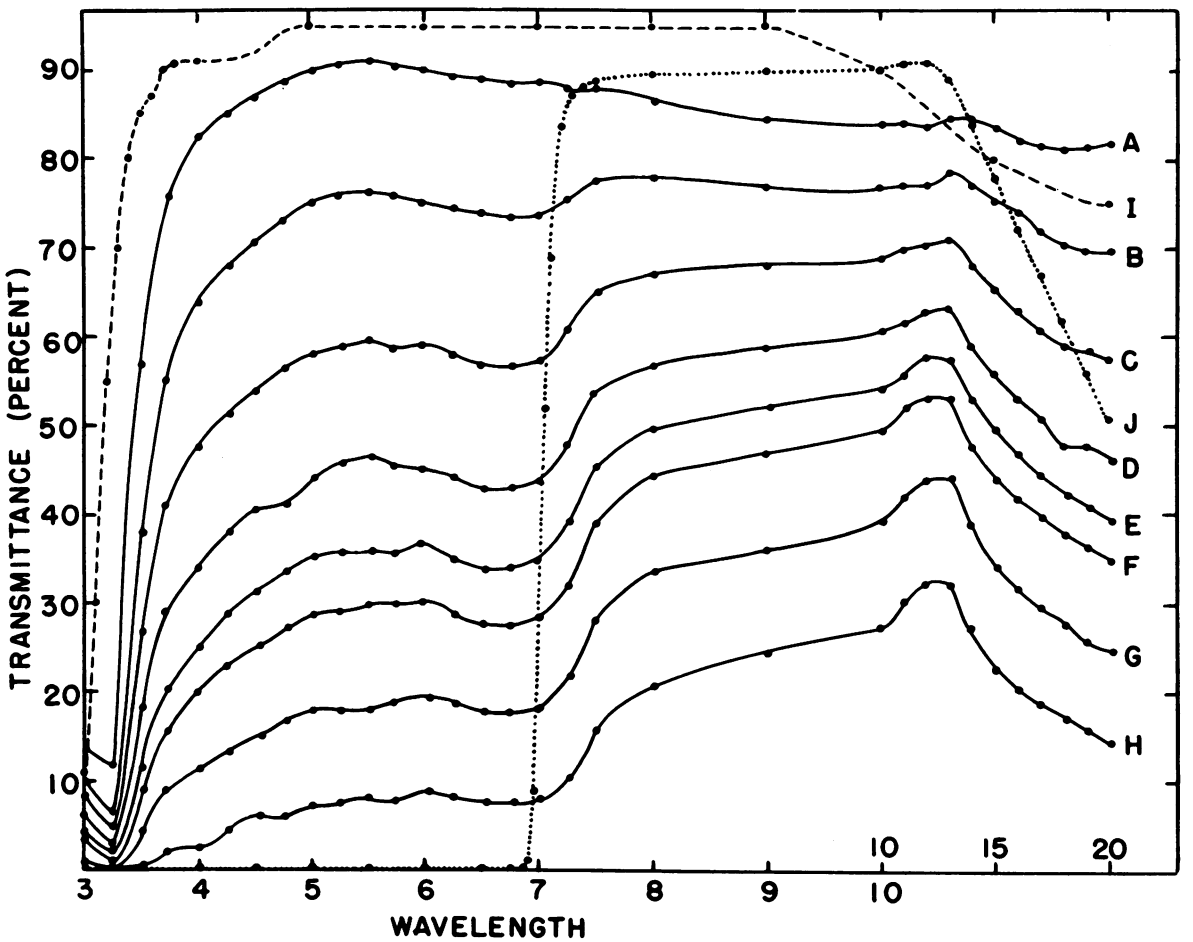

FIG. 1. Transmission curves of the filters used. A–H, the series of Wratten neutral filters. I, the thermopile window. J, the RG8 filter. The unit of wave length is 10^3 Å.

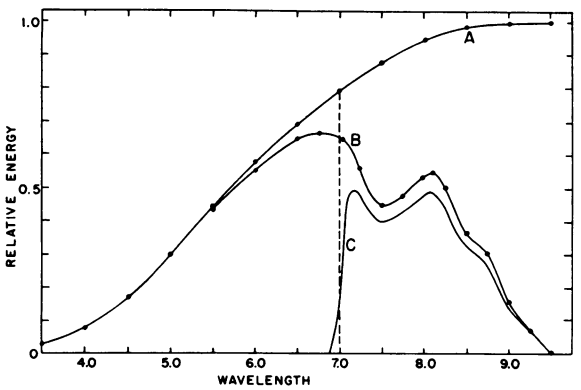

FIG. 2. Spectral characteristics of the illumination. A, the relative energy output of the 1000-watt lamp at 100 volts. B, the relative energy passed by 23.5 cm water. C, the relative energy passed by 23.5 cm water plus the RG8 filter. The unit of wave length is 10^3 Å.

the area under curve C includes a small part of the visible region and excludes about 7 % of the infrared. The net effect is that the visible intensity as estimated is about 105 % of the true value. This error is almost exactly balanced by the window correction for the thermopile which was calibrated without a window but used with a window of 95 % transmission (fig 1). A more serious intensity error lies in reflection losses at the air-lucite-water and water-lucite-water interfaces of the growth chamber. The net transmission of the front surface of the growth chamber into the algal suspension was estimated at 85 % by differential measurements on a lucite absorption cell using a Beckman spectrophotometer. In the intensity data to be presented, none of the above corrections has been applied since they are entirely constant throughout the series of measurements.

INTERMITTENCY CHARACTERISTICS: Intermittency of illumination was obtained by rotating sectors. A series of aluminum disks 1/16″ thick and 40″ circumference were cut to give sector openings of 1/4″, 1″, or 4″ as measured at the periphery. A disk in use was mounted on the shaft of a gear ratio box coupled directly to a 1/5 HP synchronous motor. A complete description of important characteristics of the sector disks used is presented in table I.

The position of a sector disk in the optical system (fig 1, S, of the preceding paper) is such that it cuts the uniform emerging beam from the condensing lens, and its image is sharply focused upon the center plane of the growth chamber. As a sector opening crosses the light beam its transmitted bar of light sweeps across the growth chamber.

For simplicity of interpretation of the end results it is desirable that the light flashes seen by each algal cell are sharply defined so that a plot of intensity against time takes a square-wave form. Some small and unmeasurable error is introduced since the algal cells are themselves moving erratically in the stirred suspension. For any one sector and rpm the lengths of a light flash as seen by different cells probably falls on a distribution curve with mean value at the calculated length (table I). A second type of error, due to lens aberrations and the scattering by the suspension itself, causes a smearing of the square-wave form. The time characteristics of a light flash have been estimated by a simple method. With the disk at rest and an edge of a sector opening in a vertical position, a photocell with a vertical 0.25 mm diaphragm was moved horizontally across the edge of the light field in the plane normally occupied by the growth chamber. Galvanometer deflections were recorded as a function of horizontal position of the diaphragm and the data are presented as curve A of figure 3. The procedure was repeated with a typical algal suspension in an absorption cell 0.5 cm thick (curve B) and again in a cell 1.0 cm thick (curve C) placed just in front of the diaphragm. The intensity distribution in light flashes produced by 1/4″ and 1/2″ sectors may be visualized from the completed curves of figure 3. Curves A, B, and C represent the light flash contours occurring at the front surface, the center, and the back surface of the algal suspension.

While figure 3 presents an adequate description of the light flashes, it does not completely characterize the dark periods. Of critical importance is the integrity of the darkness maintained during the dark periods, particularly when these are very long, for reasons which will be considered in the subsequent discussion. The characteristics of a typical long dark period were examined by the following procedure. The growth chamber was replaced by a photocell receiver faced with a diaphragm of 3 mm diameter and a ground glass plate. A sector with four 1″ openings (Experiment 73, table III) was rotated manually and galvanometer deflections were recorded as a function of peripheral movement. The larger diaphragm opening gives poorer resolution of the edge of the flash but allows more accurate estimation of light leakage

TABLE I

SECTOR CHARACTERISTICS

EXPT.	SECTOR SIZE	OPENINGS	DISK SPEED	t_f	t_d	$\frac{t_f}{t_f + t_d}$	FREQ.
no.	*in.*		*rpm*	*sec*	*sec*		*sec*$^{-1}$
82	4	1	90	0.0667	0.600	0.100	1.5
81	4	2	90	0.0667	0.266	0.200	3
80	4	4	90	0.0667	0.100	0.400	6
75	1	2	90	0.0167	0.316	0.050	3
73	1	4	90	0.0167	0.150	0.100	6
74	1	8	90	0.0167	0.067	0.200	12
77	1	12	90	0.0167	0.039	0.300	18
66	1	1	360	0.00417	0.163	0.025	6
67	1	2	360	0.00417	0.079	0.050	12
65	1	4	360	0.00417	0.037	0.100	24
68	1	8	360	0.00417	0.017	0.200	48
78	1	12	360	0.00417	0.010	0.300	72
70, 72	1/4	4	360	0.00104	0.041	0.025	24
69	1/4	8	360	0.00104	0.020	0.050	48
71	1/4	16	360	0.00104	0.009	0.100	96
79	1/4	24	360	0.00104	0.006	0.150	144

during the dark period. The data are presented in figure 4.

CHLOROPHYLL CONCENTRATION OF THE CELLS: At the termination of each growth experiment two supplementary determinations were made on the final culture suspension. Relative chlorophyll concentration was determined by a Beckman spectrophotometer on a 10 ml solution obtained by extracting the cells from a 5.0 ml aliquot of suspension in boiling methanol. Cell volume was determined by centrifuging aliquots of suspension to constant packed volume in calibrated capillary tubes.

with increasing intensity. An approximate value of intensity for light saturation may be estimated at 2.5×10^4 erg/cm^2-sec where the growth rate attains about 90 % of its observed maximum. This value, corresponding to about 600 fc, is considerably higher than the 100 fc which can be obtained from a similar curve of Myers (11). The present value of 600 fc was obtained under unilateral illumination; the earlier value of 100 fc was obtained in an annular chamber illuminated by four tubular lamps and the reflections from a surrounding housing. The discrepancy illustrates an error easily made in the design and interpre-

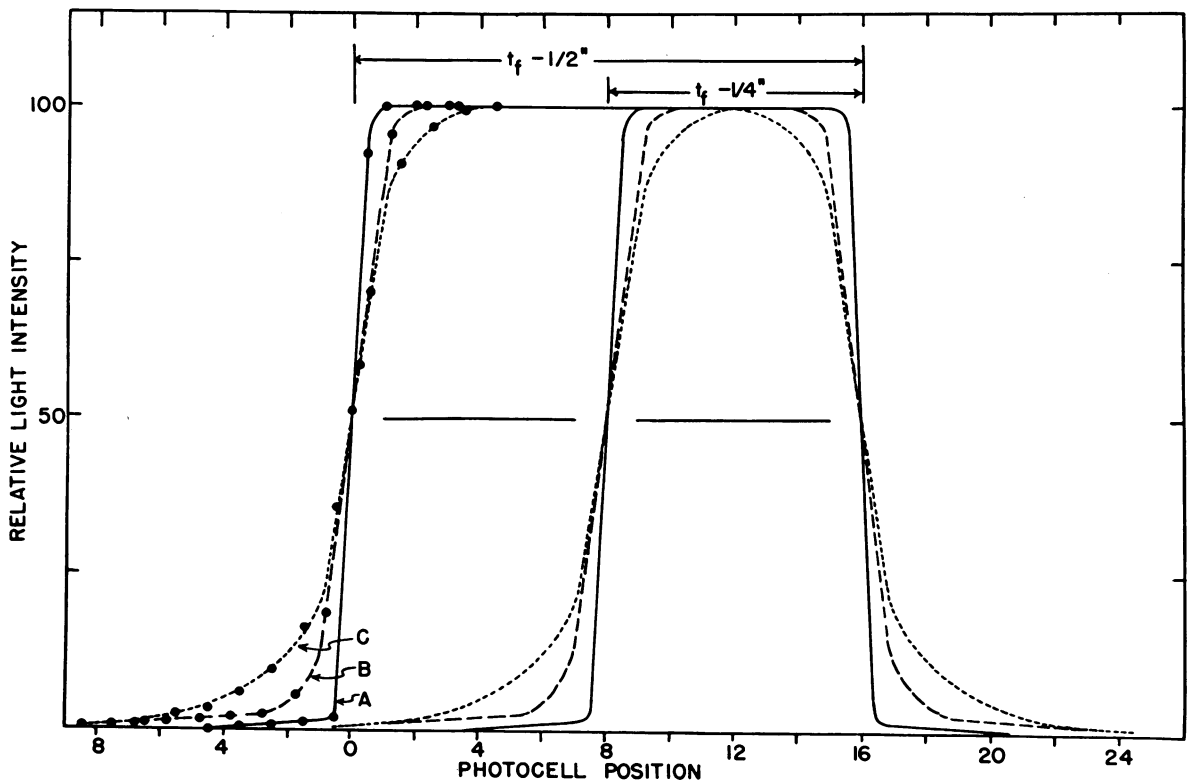

FIG. 3. Time characteristics of the light flash as seen by algal cells at the front surface (A), the center (B), and the back surface (C), of the growth chamber. See text.

RESULTS AND DISCUSSION

CONTINUOUS ILLUMINATION: Experiments measuring the specific growth rate as a function of intensity of continuous illumination are summarized in table II. The data are plotted in figure 5 as solid points connected by a solid line to form the expected saturation curve. There is a small anomaly in the points at very low light intensity which appear to cause a break in the curve. However, since small values of the growth rate are established with lowered precision, no significance is attached to the apparent anomaly. The extrapolated value of the compensation point for growth can be estimated only as a value less than 1000 erg/cm^2-sec or 24 fc.

At high light intensities, growth rate is not completely light-saturated but continues to increase slowly

tation of experiments concerned with evaluation of light intensity effects. Measurements of critical values of intensity made under multi-directional illumination do not provide data which submit to general interpretation. Error of interpretation arising from these considerations was made by Myers (11) and has been made by others in interpretation of the results of Hoover, Johnston, and Brackett (7) on wheat leaves obtained under similar conditions of multi-directional illumination.

INTERMITTENT ILLUMINATION: The series of experiments on growth rate as a function of intermittency are summarized by table III. Characteristics of intermittency in each experiment are defined by the flash time, t_f, the dark time, t_d, and the fractional time in the light, $t_f/(t_f + t_d)$, the initial light intensity, I_o, and the integrated light intensity, I_a, on the growth

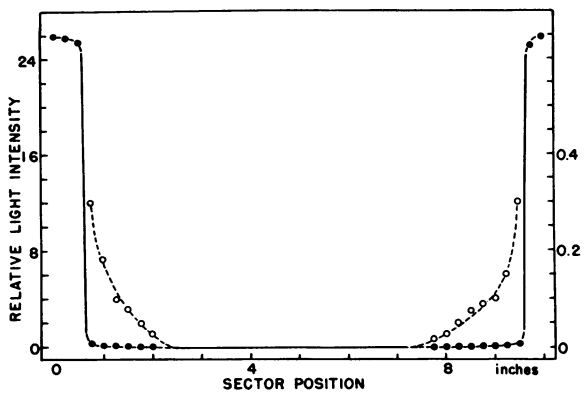

FIG. 4. Time characteristics of the dark period as determined using a disk with four 1" sector openings (experiment 73). Relative light intensity (galvanometer readings) versus position of the disk measured at the periphery. ●——● original data, left scale; ○- - -○ original data × 40, right scale.

chamber. It was desired that I_o (measured with the sector removed) be kept constant throughout all experiments but in practice this value varied ± 7 % from a mean of 228×10^3 erg/cm²-sec, owing to lamp decay and variation between lamps. The integrated intensity, I_a, was calculated as $I_o \times t_f/(t_f + t_d)$ and measured directly by the thermopile with the sector in operation. Values of I_a obtained by the two methods are not in very good agreement. The disk of experiment 79, which showed the most severe discrepancy, was carefully checked with a cathetometer. Individual openings showed variations of ± 12 % in width and area and a total open area of 0.749 of the nominal open area ($t_f/(t_f + t_d)$ of table I). The I_a (measured) of table III is 0.751 of the I_a (calculated). Only the measured values of I_a are used in the subsequent treatment.

Effects of intermittent light on photosynthesis and growth can be treated as a problem in light integration. If photochemical intermediates produced in a short flash of high intensity can be processed further by enzymatic reactions during the following dark period, then the cell is accomplishing a time integration of the light energy received. Following this line of argument, as proposed by Kok (8), growth rates in intermittent light have been plotted in figure 5 against integrated intensity (I_a, measured, table III). The resulting curves A, B, C, D have parameters of flash time t_f. A base line, (X), may be drawn for the limiting case in which reactions leading to growth occur at maximum rate during the light flashes but not at all during the intervening dark periods, ie, for zero integration. Calculation of values on curve X are made simply as $2.1 \times t_f/(t_f + t_d)$ where 2.1 is taken as the maximum growth rate in continuous light.

Comparison of the curves of figure 5 gives a measure of the degree to which the alga integrates intensity when light of high intensity (~ 230×10^3 erg/cm²-sec) is presented in flashes of various lengths. If integration is perfect, a curve for intermittent light will superimpose the curve for continuous light. It will be seen that this condition is closely approached when flashes of 0.001 second are used. As the flash time is increased (curves C, B, A) the degree of integration becomes lower and the curves approach the base line, X, of zero integration. An unexpected character of curves B, C, and D is their approach toward higher growth rates than those observed at equal integrated intensities of continuous light.

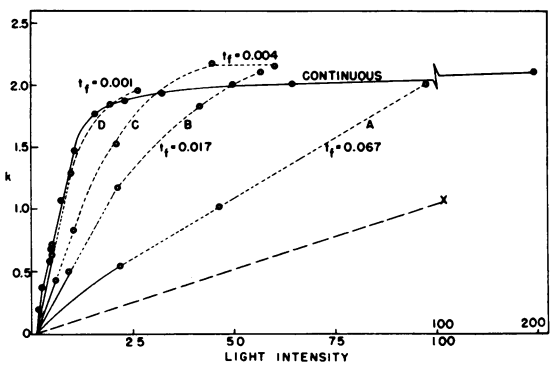

FIG. 5. Growth rate versus light intensity. Solid line: in continuous illumination (table II). Broken lines: in intermittent light (table III) for the flash time noted on each curve and with the integrated intensity determined by the length of the dark period. The unit of light intensity is the kiloerg/cm²-sec.

TABLE II

GROWTH RATE AS A FUNCTION OF INTENSITY OF CONTINUOUS ILLUMINATION

EXPERIMENT	INTENSITY	k
no.	*erg/cm²-sec* × 10^3	*log$_e$ units/day*
60	1.54	0.20
56	2.12	0.37
61	4.10	0.58
58	4.55	0.68
57	7.63	1.07
55	11.2	1.48
59	16.0	1.77
62	20.3	1.88
54	26.4	1.92
76	49.7	2.00
64	65.3	2.01
63	196.0	2.10

A second and more conventional treatment of flashing light data is based on yield per flash as a function of dark time, following the argument of Emerson and Arnold (5). In measurements of photosynthesis yield per flash may be expressed in μl $O_2/\mu l$ cells per flash. The specific growth rate k (as will be demonstrated below) has dimensions equivalent to μl $O_2/\mu l$ cells per unit time. Growth yield per flash may be expressed in terms of k/flash, as in the ninth column of table III. The data are plotted in figure 6 to give a family of curves of flash yield against dark time for the four values of t_f used. As t_d approaches zero, the yield per flash approaches the value of k/t_f observed in continuous illumination at high light intensity. This limit yield per flash at zero t_d was negligible for the very short flashes (10^{-5} sec) used by Emerson and Arnold. In the present experiments,

TABLE III

GROWTH IN INTERMITTENT ILLUMINATION

EXPT.	t_f	t_d	I_o	I_a (CALC.) *	I_a (MEAS.)	k	GAIN-k **	k †	GAIN-k
no.	*sec* $\times 10^{-3}$	*sec* $\times 10^{-3}$	*erg/cm²-sec* $\times 10^{3}$	*erg/cm²-sec* $\times 10^{3}$	*erg/cm²-sec* $\times 10^{3}$	*day*$^{-1}$	*day*$^{-1}$	*flash*$^{-1} \times 10^{-7}$	*flash*$^{-1} \times 10^{-7}$
82	66.7	600	218.0	21.8	21.5	0.54	0.33	41.7	25.5
81	66.7	266	226.5	45.3	46.5	1.20	0.78	46.3	30.1
80	66.7	100	241.2	96.5	97.3	2.01	1.17	38.8	22.6
75	16.7	316	211.1	10.6	9.88	0.50	0.40	19.3	15.4
73	16.7	150	221.1	22.1	21.2	1.18	0.97	22.8	18.7
74	16.7	67	222.5	44.5	42.1	1.83	1.41	17.7	13.6
77	16.7	39	212.2	63.7	57.4	2.11	1.48	13.6	9.52
66	4.2	163	237.6	5.94	5.75	0.42	0.37	8.10	7.14
67	4.2	79	239.5	12.0	10.4	0.83	0.72	8.01	6.94
65	4.2	37	228.9	22.9	21.7	1.53	1.32	7.38	6.37
68	4.2	17	239.6	47.9	44.5	2.18	1.76	5.26	4.24
78	4.2	10	225.6	67.7	60.4	2.15	1.52	3.46	2.44
70	1.0	41	222.3	5.56	4.83	0.63	0.58	3.04	2.80
72	1.0	41	231.8	5.80	4.97	0.71	0.66	3.42	3.18
69	1.0	20	223.5	11.2	9.54	1.29	1.18	3.11	2.85
71	1.0	9	230.8	23.1	19.4	1.84	1.63	2.22	1.97
79	1.0	6	241.2	36.2	27.2	1.96	1.64	1.58	1.32

* $I_o \times \frac{t_f}{t_f + t_d}$.

** $k - \frac{t_f}{t_f + t_d} \times 2.10$.

† $\frac{k}{\text{flashes/sec} \times 8.64 \times 10^4 \text{ sec/day}}$.

however, the limit yield at zero t_d is not negligible for the longer light flashes. The curves of figure 6 describe not only the effects of intermittency but also the effects of continuous illumination operating during the significantly long periods of the light flashes. The latter effects may be eliminated by subtracting the value of k on curve X of figure 5 from the observed value of k in flashing light of the same integrated intensity. This procedure has been applied to give the data, labelled "gain-k" in the eighth column and "gain-k/flash" in the tenth column of table III, which characterize only the intermittency effects. Actually the procedure makes some degree of overcorrection; it is based on the assumption that the photochemical apparatus is maintained at a constant degree of light saturation throughout the length of the light flash and independent of the length of the preceding dark period.

Yield per flash in terms of gain-k is plotted in figure 7 as a function of t_d. With increasing dark times the flash yields approach maximum values. (No explanation is apparent for the somewhat decreased flash yields observed for the longer flash times and very long dark periods and no particular significance is attached to this aspect of the data.) The maximum flash yield (and also the dark time required to obtain half-maximum flash yield) show a saturation curve when plotted against t_f, as in the insert of figure 7.

Comparison of the present data on specific growth rate with those of photosynthesis studies requires conversion of k to the units measured by manometry. When k = 1.0 $\log_e$ unit per day, 1.0 μl cells will produce 1.0 μl of new cells per day. 1.0 μl of cells, determined as the minimum packed volume on centrifugation, is equivalent to 0.24 mg dry weight and 120 μgm or 10 μmols of carbon. The equivalents are based upon previously published values from this laboratory and show only minor variation with light intensity. Ten μmols of carbon require an uptake of 224 μl carbon dioxide. Conversion to oxygen evolved is attended by some uncertainty as to the proper choice of assimilatory quotient. At low light intensities and with nitrate as the nitrogen source, the quotient

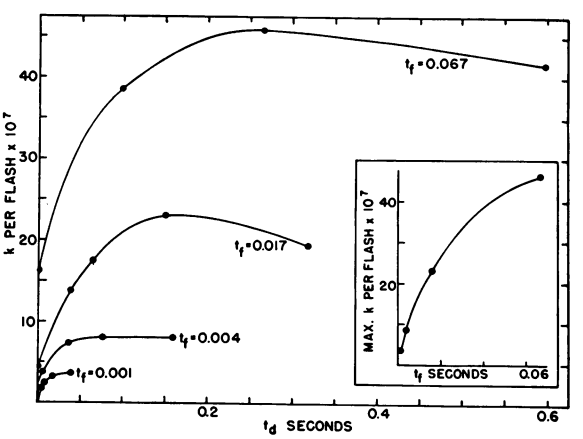

FIG. 6. Flash yield in terms of k/flash as a function of dark period for each flash time.

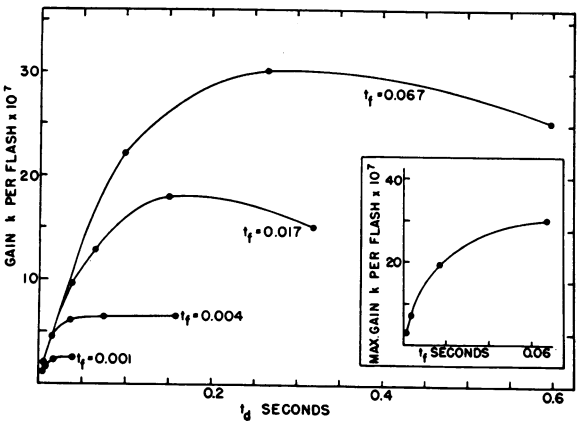

FIG. 7. Flash yield in terms of gain-k/flash as a function of dark period for each flash time. For definition of "gain-k" see text.

CO_2/O_2 has been shown to be 0.7 (4). Unless there are severe changes in nitrogen content the quotient during steady-state growth at high light intensities will be 0.7 also; at any rate it will not be higher than 0.9 as measured in short-time manometric experiments. For present purposes 224 μl carbon dioxide are taken as equivalent to 320 μl O_2 (A.Q. = 0.7) as the more likely value although it is recognized that the equivalence might be as low as 250 μl O_2 (A.Q. = 0.9). In short, values of k may be converted to μl O_2/μl cells upon multiplying by 320.

Comment should be made at this point that chlorophyll content does not enter in any way into the determination of any one value of k. Each value of k was determined during steady-state growth under conditions of constant chlorophyll content. It is true that the photometric method used is sensitive to chlorophyll absorption as well as to light scattering. However, even if the photometric device responded only to chlorophyll so that the method directly measured k in terms of Δ chlorophyll/chlorophyll per unit time, then within any one determination on a given steady-state the same value of k would describe also Δ cell number/cell number and Δ cell weight/cell weight per unit time. A quite different consequence of chlorophyll content is discussed in a later section.

The present data are compared in table IV with those obtained in other investigations. The important value of maximum flash yield shows a 27-fold variation and the minimum dark time required for maximum yield shows a 13-fold variation. Most of the variation is introduced by the longer flash times used by Tamiya (15) and in the present study and obtained by the use of rotating sectors. There are three possible interpretations. (1) Use of sectors and higher values of t_f and t_d is attended by some systematic error. (2) True flash saturation has not been achieved by different workers but is more closely approached by the use of longer flash times obtainable with sectors. (3) The use of longer flash times introduces new phenomena dependent upon different reactions from those which govern the response to very short flashes. Comment will be made on all three possibilities.

Chopping of a high intensity beam from a continuous source may give rise to serious difficulties if there is light leakage during the dark periods. In the present case the question was raised whether the higher flash yields observed with longer light flashes

TABLE IV

COMPARATIVE DATA FROM FLASHING LIGHT INVESTIGATIONS

WORKERS *	TEMP.	MAX. RATE CONTINUOUS ILLUMINATION	t_f	t_d MINIMUM FOR MAX. YIELD	t_d FOR YIELD CITED	MAX. FLASH YIELD	REFERENCE
	°C	$\mu l\ O_2/hr \cdot \mu l$	$sec \times 10^{-3}$	$sec \times 10^{-3}$	$sec \times 10^{-3}$	$\mu l\ O_2/\mu l$ per flash $\times 10^{-5}$	
Emerson & Arnold (5)	24.9	21	0.01	<35	>35	5.0	Protocols I & II
Emerson & Arnold (6)	24.9	..	0.01	..	48	10.0	Table I
high chlorophyll	24.9	37	0.01	..	83	16.0	Table II
low chlorophyll	24.9	6	0.01	..	83	3.5	Table II
Clendenning & Ehrmantraut (2)	10	..	0.01	40	100	5.2	Fig 8
Weller & Franck (16)	20	18	4.2	62	>62	13.0	Fig 2
Rieke & Gaffron (14)	20	(18) **	4.2	..	58	8.3	Table I
Tamiya (15)	25	31	8.0	150	300	40.0	Protocol IIId
	25	31	3.0	120	200	29.0	Protocol IVc
	25	31	1.0	70	200	14.0	Protocol IVb
Present data	25	28	67.0	266	266	96.0	Exp 81
	25	28	17.0	150	150	60.0	Exp 73
	25	28	4.2	40	163	23.0	Exp 66, 67
	25	28	1.0	20	41	9.6	Exp 70, 72

* The results of Kok (8) are omitted only because of lack of tabulated data.
** Value assumed equal to that observed by Weller & Franck.

could be due to a lack of integrity of the very long dark periods required. Check upon this point was made with the sector giving maximum flash yield for $t_f = 0.017$ second (experiment 73 and figure 4). From the data of figure 4 it may be seen that the middle one half of the 0.150 second dark period is entirely dark; the remaining one half includes the tails from the light flashes which summate to an average intensity equivalent to 600 erg/cm²-sec operating over 0.45 of the total time ($t_f + t_d$). From the continuous illumination data it may be estimated that the intensity leakage in this particular regimen could not have caused an error in k of more than 0.08. A more important result, however, is that light leakage during the dark period is due only to the tails of the light flashes. The higher yields at longer light flashes and dark periods cannot be attributed to light leakage during the dark periods.

The question of attainment of flash saturation in the experiments of Emerson and Arnold (5, 6) has been examined by several workers with opposing conclusions. Tamiya (15) obtained variable t_d, t_f, and flash intensity by sector chopping of a continuous source. Maximum flash yield was obtained only with high flash energies which required long flash times and very long dark periods of about 0.2 second at 25°C. Further, the maximum flash yield was temperature dependent except at very low flash energies. Tamiya did not apply any correction for the amount of photosynthesis occurring during his long light flashes; recalculation of his data and introduction of such a correction does not make any important change in his results. Tamiya concluded that the short dark periods and other results of Emerson and Arnold were consequences of lack of attainment of flash saturation.

Weller and Franck (16) reinvestigated the question of flash saturation by use of a sector synchronized with the inherent intermittency of a mercury arc to give flashes of 0.0042 second and various dark times. Clendenning and Ehrmantraut (2) and Ehrmantraut and Rabinowitch (4a) also have presented pertinent data obtained with a neon tube flashed by a condenser discharge to give flashes of about 10^{-5} seconds similar to those of Emerson and Arnold. These studies contain various types of evidence for attainment of flash saturation in very short flashes. Their results were interpreted as supporting the argument of Emerson and Arnold that the maximum yield produced by a very short and saturating flash is determined only by the quantity of an enzyme with a turnover time of about 0.01 second at 25°C. However, no study yet made with very short flashes provides conclusive evidence for both flash saturation and temperature-independence of maximum yield which is necessary to refute Tamiya's conclusion.

A third possible explanation of the differences between short-flash and long-flash intermittency results has been suggested by Weller and Franck (16) and by Ehrmantraut and Rabinowitch (4a). When the light flash and dark period are lengthened, the limitation controlling flash yield is transferred from the "Emerson and Arnold enzyme" to some other slower enzyme system. The new system may be the one responsible for the dark pick-up phenomenon observed by McAlister (10). This is not a completely satisfying explanation. The experimental observation is that longer light flashes not only require longer dark periods, but also give higher flash yields even when the flash yield is corrected by subtracting the maximum amount of photosynthesis which could occur during the flash itself. It is a straightforward argument that lengthening of the light flash may lead to longer dark periods required by limitations of a second enzyme system. It is not at all apparent how the transfer to the second and apparently more restricting limitation can increase the flash yield.

The present data on growth rate in intermittent light have about the same precision as the manometric data of other investigations with which they have been compared. They may be compared with data on photosynthesis since the critical values for maximum flash yields and dark periods are obtained at less than the maximum growth rate where photosynthesis must be the growth-limiting process. They support the results of comparable sector experiments of Tamiya (15) and Kok (8) that long flash times require long dark periods of tenths of a second to obtain maximum flash yields. Comparison with the results of short flash experiments such as those of Emerson and Arnold (5, 6) requires either that light saturation is not attained in the short flashes or that some new limiting phenomenon is introduced by the use of longer flashes. In passing, it may be noted that none of the flashing light data support the very long dark period of the order of minutes postulated on theoretical grounds by Burk, Cornfield, and Schwartz (1).

Chlorophyll Content: Methanol extracts of the cells harvested at the end of each growth experiment showed no significant differences in the shape of the absorption curve determined between 6000 and 7000 Å. The peak of the curve at 6700 Å has been used as a measure of chlorophyll concentration. Data expressing relative chlorophyll concentration per unit volume of cells are plotted in figure 8. Chlorophyll content is the same function of light intensity previously reported by Myers (11). In intermittent light the data show the same pattern with minor deviations when plotted in terms of integrated light intensity. Unfortunately, the small quantity of cells available in each experiment limits the precision of the chlorophyll assay, and conversion to absolute amounts is not merited. Use of MacKinney's (9) absorption coefficient of 74.5 for chlorophyll in methanol at the red peak gives chlorophyll contents within the range observed by Emerson and Arnold (6).

The variability in chlorophyll concentration with light intensity means that the concentration contributes to the characteristics of the light intensity curve for growth (fig 5). The slope of the light-limited region of the curve depends upon two factors: the fractional absorption of light per cell and the quantum efficiency for growth. In analogous measurements of the light intensity curve for photosynthesis the curve is commonly determined in short-time experiments

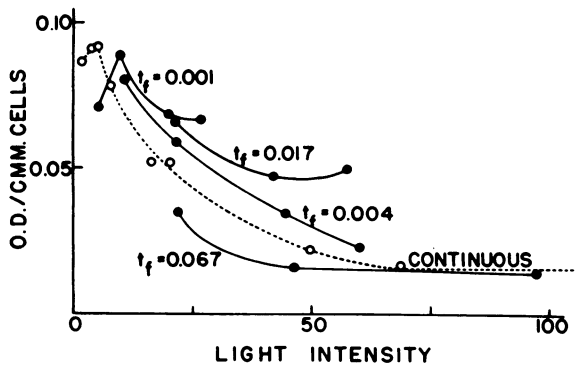

FIG. 8. Chlorophyll content of the cells as a function of light intensity at which they were grown. Relative chlorophyll content expressed in optical density per μl cells as determined at 6700 Å for a 10 ml methanol extract in a 1.0 cm cuvette. The unit of intensity is the kiloerg/cm^2-sec.

with cells of constant chlorophyll content and, hence, constant light absorption per cell. In long-time measurements of growth rates the chlorophyll content and the light absorption per cell are independent functions of light intensity. Hence any growth response to light intensity or intermittency includes the effects of intensity or intermittency upon chlorophyll concentration.

PRACTICAL ASPECTS: The entirely practical question, toward which the present work was directed, is the possible contribution of turbulence of culture to increase in growth rate in dense cultures. The light intensity of 23×10^4 erg/cm^2-sec used is about 0.6 of the maximum intensity of full sunlight in the visible region. It can be seen from the curves of figures 5 and 6 that a dense culture growing under sunlight will experience a significant increase in growth if cells are moved in and out of the high intensity of the front surface at such a rate as to give flash times between 0.001 and 0.1 second. It is also clear that the culture should be thick enough or dense enough so that almost all the light will be absorbed in the first 10 % and the dark time will be about ten times as long as the light flash. These considerations lead to the conclusion that almost any attempt to grow algae in sunlight will experience some gain by turbulence. The feasibility of increasing the turbulence will depend upon the extent of the gain in growth as compared to the increased power requirement of stirring or pumping the suspension.

SUMMARY

Growth rate of the alga, *Chlorella pyrenoidosa*, has been measured at 25°C, as a function of intensity and intermittency of illumination. The saturation curve obtained in continuous light shows a compensation point of less than 1000 erg/cm^2-sec or 24 fc and an approximate saturation point at 25×10^3 erg/cm^2-sec or 600 fc.

Intermittent light was obtained by sector chopping of a beam of 230×10^3 erg/cm^2-sec to give light flashes of 1, 4, 17, and 67 milliseconds and various dark periods. With one millisecond flashes the alga almost completely integrates intensity times time so that growth response to integrated light intensity is the same as that observed in continuous illumination. With longer flashes the degree of integration decreases but remains significant even at 67 milliseconds.

The intermittent light data have been treated also in terms of flash yield and compared with those of investigations on photosynthesis. Comparison of the critical data of the several investigations reveals a range of variation which is not explainable in terms of the classical arguments used to explain the intermittency phenomena observed with very short flashes.

The results allow prediction that partial, but probably not complete, advantage of the intermittency phenomenon may be taken to increase the efficiency of light utilization of an algal culture by turbulence of suspension.

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RECORDING OXIDATION-REDUCTION POTENTIALS IN PLANT PREPARATIONS[1,2]

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The study of oxidation-reduction potentials often represents a powerful tool in biological and chemical investigations (1). This paper describes simple techniques for measuring and recording oxidation-reduction potentials in photosynthetic systems. The methods described could easily be adapted to measurements on other biological and chemical systems.

Isolated chloroplasts of higher plants suspended in solutions of suitable electron acceptors (oxidants) such as ferricyanide carry on reactions of the following type upon illumination:

(1) $4\ \text{ferricyanide} + 2\ H_2O \rightarrow 4\ \text{ferrocyanide} + 4\ H^+ + O_2$

This reaction, usually called the Hill reaction (2) apparently represents the energy-absorbing and water-splitting part of photosynthesis. A number of useful techniques have been developed for studying this reaction including the measurement of oxygen evolution (2, 3), the measurement of hydrogen-ion formation (3), and the measurement of rate of decolorization of oxidation-reduction indicators (4). The Hill reaction has been studied in this laboratory by determining the rate of reduction of added electron acceptors potentiometrically (5). Advantages of this method include (a) increased precision of measurement (± 1 %), (b) system volumes of less than 0.25 ml, (c) use of oxidant concentrations as low as 10^{-6} M, (d) automatic recording of results, and (e) use of reaction cells with simple optics.

MATERIALS AND METHODS

RECORDING OF OXIDATION-REDUCTION POTENTIALS: The output of a platinum-calomel electrode system was fed through an impedance matching device into a Brown "Electronik" Single-Record Strip Chart Recorder with a self-contained amplifier. Two models were used successfully: a Series 153X11 with a pen speed of 12 seconds for full scale travel (11 inches) and a range of 0 to 2.5 mv, and a Series 153X18 with a pen speed of 1 second and a range of 0 to 1 mv. An impedance matching circuit was necessary since the input impedance of the recorder was 1000 ohms while the electrode system required a high impedance circuit to avoid polarization effects. A typical two-tube cathode follower with a bias circuit to match the tubes was used as shown in figure 1. This seemed ideal since it (a) is extremely stable, (b) shows low sensitivity to changes in plate voltage, (c) shows very low distortion, (d) has a high input impedance (estimated at 50 megohms), and (e) has a low output impedance to match the recorder. The tubes were aged before use, and stability was further increased by using low filament and plate voltages. Unsuccessful attempts were made to use an alternating cur-

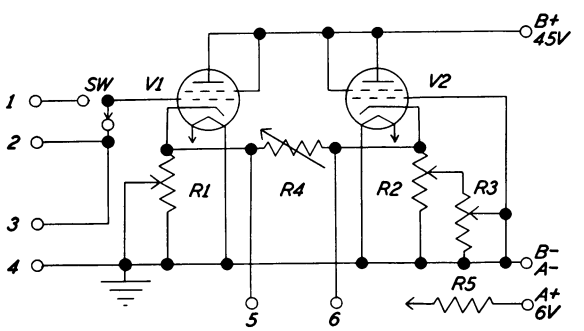

FIG. 1. Battery operated, cathode-follower, input device to match the high impedance of a platinum-calomel electrode system to the low input impedance of a Brown recorder.

V1, V2: 6K6GT radio tube.
R1, R2, R3: 10,000 ohm wire-wound potentiometer for balancing the circuit.
R4: decade resistance box to control sensitivity.
R5: 2 ohm wire-wound filament dropping resistor.
1, 2: to electrode system.
3, 4: to potentiometer for calibration and bucking voltage.
5, 6: to input of Brown recorder.
SW—SPDT: switch to connect electrode system into the input circuit.

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